

CLAIMS

1. A method of chemical analysis comprising the steps of  
combining one test compound with a solution comprising m enzymes and n  
5 substrates complementary to said enzyme, wherein m is an integer equal  
to one or greater, n is an integer equal to one or greater, and  $m + n \geq 3$ ,  
incubating for a period of time said test compound within said solution,  
separating the chemical species in said combined solution by a  
chromatography step after said incubating step, and  
10 measuring the relative amounts of substrates and separately identifiable  
products produced therefrom by a chemical reaction catalyzed by said  
enzymes  
wherein said chromatography step is carried out within a microfluidic device.
- 15 2. The method of claim 1 further comprising the step of comparing the data  
obtained in the measuring step to data collected from repeating the method of  
claim 1 under substantially identical conditions but with a different test  
compound.
- 20 3. The method of claim 1 further comprising the step of comparing the data  
obtained in the measuring step to data collected from repeating the method of  
claim 1 under substantially identical conditions but with no test compound.
4. The method of claim any one of claims 1 – 3 wherein  $m = 1$  and  $n \geq 2$ .
- 25 5. The method of claim any one of claims 1 – 3 wherein  $m \geq 2$  and  $n = 1$ .
6. The method of claim any one of claims 1 – 3 wherein  $m \geq 2$  and  $n \geq 2$ .
- 30 7. The method of claim 1 wherein said chromatography step is electrophoresis or  
ion chromatography; high, medium, or low pressure liquid chromatography; or  
any combination thereof.

10027922 "1.22101

8. The method according to claim 7 wherein said chromatography step is capillary electrophoresis.
- 5 9. The method according to claim 8 wherein said enzyme catalyzes a reaction in which the products have a different mass to charge ratio than the substrates.
10. The method according to any one of claims 8 or 9 wherein said products may be resolved in a capillary electrophoresis column of less than 8 cm in length in  
10 under about 5 minutes.
11. The method according to claim 1 wherein said enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase, or ligase.
- 15 12. The method according to claim 1 in which the combining and incubating steps of claim 1 are multiply and nearly simultaneously or sequentially executed.
13. The method according to claim 1 wherein said microfluidic device further comprises a reaction means in which said incubating step is executed.
- 20 14. The method according to claim 1 wherein  $m \leq 50$ .
15. The method according to claim 14 wherein  $m \leq 10$ .
- 25 16. The method according to claim 1 where in  $n \leq 50$ .
17. The method according to claim 16 wherein  $n \leq 10$ .
18. The method according to claim 1 wherein said test compound reduces the rate at  
30 which said enzyme converts said substrate into said product.

19. The method according to claim 1 wherein said test compound increases the rate at which said enzyme converts said substrate into said product.
20. The method according to claim 1 wherein said chemical reaction is a hydrolysis, oxidation-reduction, metathesis, or isomerization reaction.
21. The method according to claim 1 wherein said measuring step is spectrometry or spectroscopy.
22. The method according to claim 1 wherein the physical parameter which is measured is molecular mass, chromatographic retention time, spectroscopic absorbance or emission, refractive index, electrical conductivity, or radioactivity.
23. The method of claim 13 wherein said microfluidic device comprises one or more introduction means through which solutions are placed into said microfluidic device, one or more chromatography means, and one or more reaction means within which said incubation step is executed.
24. The method according to claim 23 wherein either of said introducing means is selected from a micropipette, a capillary, a virtual wall, or an aperture.
25. The method of claim 24 wherein said introduction means is a virtual wall.
26. The method of claim 23 wherein said reaction means is a reservoir or a channel.
27. The method according to claim 1 wherein said measurement step produces data which are indicative of the thermodynamics or kinetics of said chemical reaction.
28. The method according to claim 27 wherein said data are collected for reactions occurring at different temperatures or concentrations.

29. The method according to claim 27 wherein the molar concentration of said enzyme is different than the molar concentration of said substrate or test compound.
- 5 30. The method according to claim 1 wherein said enzyme is a synthetase, protease, esterase, kinase or phosphatase.
31. The method according to claim 1 wherein said substrate is the naturally occurring substrate of said enzyme, or a fragment thereof.
- 10 32. The method according to claim 1 wherein said substrate is a nonnatural or synthetic substrate for said enzyme.
33. The method according to any one of claims 31 or 32 wherein said substrate is covalently bonded to a chromophore.
- 15 34. The method according to claim 1 wherein said test compound is a member of a combinatorial library.
- 20 35. The method according to claim 1 wherein said test compound has a molecular weight of less than 2500.
36. The method according to claim 35 wherein said test compound has a molecular weight of less than 1500.
- 25 37. The method according to claim 1 wherein said test compound is not a peptide.
38. The method according to claim 1 wherein  $m \geq 2$  and said measuring step produces data which indicate the relative specificity of said test compound for preferentially altering the rate of said chemical reaction with respect to one enzyme-substrate pair substantially more than for the other enzyme-substrate pair
- 30

39. The method according to claim 1 wherein  $m \geq 2$  and said measuring step produces data which indicate the relative specificity of said test compound for preferentially altering the rate of reaction catalyzed by one enzyme, or binding to one enzyme, substantially in preference to other enzymes.
40. The method according to claim 1 wherein  $n \geq 2$  and said measuring step produces data which indicate the relative specificity of said test compound for preferentially altering the rate of said chemical reaction with respect to one enzyme-substrate pair substantially more than for the other enzyme-substrate pair.
41. The method according to claim 1 wherein  $n \geq 2$  and said measuring step produces data which indicate the relative specificity of said test compound for preferentially altering the rate of reaction of one substrate substantially in preference to other substrates.
42. The method according to claim 1 comprising an additional step of quenching said chemical reaction after incubating and prior to chromatography.
43. A method of chemical analysis comprising the steps of combining one test compound with a solution comprising  $m$  enzymes and  $n$  substrates complementary to said enzyme, wherein  $m$  is an integer equal to one or greater,  $n$  is an integer equal to one or greater, and  $m + n \geq 3$ , incubating for a period of time said test compound within said solution, separating the chemical species in said combined solution by a capillary electrophoresis chromatography step or capillary electrochromatography step after said incubating step, and measuring the relative amounts of substrates and separately identifiable products produced therefrom by a chemical reaction catalyzed by said enzymes

wherein said chromatography step is carried out within a microfluidic device comprising a capillary electrophoresis column.

CVZ-003-13401